



## The fate of *Robinsonia* (Asteraceae): sunk in *Senecio*, but still monophyletic?

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### Abstract

*Robinsonia* is a genus of eight species and is endemic to the Juan Fernández Islands. Previous studies based on ITS phylogenies place *Robinsonia* deeply nested within *Senecio*, however its monophyly remains uncertain. In this paper, we use phylogenies reconstructed from plastid, ITS-ETS, and combined data to test its monophyly. Plastid phylogenies support *Robinsonia* as monophyletic, whereas ITS-ETS trees suggest that *Robinsonia berteroi* may be more closely related to a South American clade of *Senecio* species rather than to the remaining *Robinsonia* species. Maximum parsimony and Bayesian inference analyses of the combined data are congruent with the plastid trees, whereas maximum likelihood analyses are congruent with the ITS-ETS data. Nodal support for either hypothesis is generally low, and Shimodaira-Hasegawa tests in which *Robinsonia* was either constrained to be monophyletic or to be non-monophyletic showed that these trees do not have significantly lower likelihood scores than trees from unconstrained analyses. Thus the monophyly of *Robinsonia* remains inconclusive despite additional data and analyses. The results of the present paper further corroborate the results of previous studies that *Robinsonia* is deeply nested within *Senecio*. We therefore propose to reduce *Robinsonia* to synonymy and present new names and combinations of the *Robinsonia* species under *Senecio*.

**Key words:** ITS, ETS, plastid, Juan Fernández Islands, incongruence, Compositae, new combinations, taxonomy

### Introduction

The Juan Fernández archipelago is composed of three small islands in the Pacific Ocean west of South America, approximately 667 km from mainland Chile (Bernardello *et al.* 2006). These islands harbor a small, but unique, flora with many endemic species. Although *Robinsonia* De Candolle in Guillemin (1833: 333, Senecioneae: Asteraceae) is comprised of only eight species, it is the second largest genus of flowering plants endemic to these islands. In previous phylogenetic studies focused on the delimitation of the genus *Senecio* Linnaeus (1753: 866, Pelsler *et al.* 2007, Nordenstam *et al.* 2009) and the intergeneric relationships within the Senecioneae (Pelsler *et al.* 2010), *Robinsonia* and four additional small genera were found to be deeply nested within *Senecio*. This finding was unexpected because *Robinsonia* is morphologically distinct from *Senecio*. All species of *Senecio sensu stricto* are monoecious herbs or small shrubs and, in contrast, *Robinsonia* species are dioecious trees or rosette shrubs. However, it is common for plant species on islands to experience strong selection pressures and become woody (Carlquist 1974, Sanders *et al.* 1987, Kim *et al.* 1996, Swenson & Manns 2003) and dioecious (Carlquist 1974, Bernardello *et al.* 2001), confounding the interpretation of evolutionary relationships using morphology alone (Kim *et al.* 1996).

In addition to being nested deeply within *Senecio*, the monophyly of *Robinsonia* remains unresolved. In an ITS phylogeny (Pelsler *et al.* 2007), five of six sampled species of *Robinsonia* formed a well-supported

clade, sister to a clade of *Senecio* species that occur in Bolivia, northern Chile, Ecuador, and Peru. However *Robinsonia berteroi* (De Candolle) Sanders, Stuessy & Marticorena in Stuessy & Marticorena (1990: 79) was placed outside the *Robinsonia* clade and was resolved as sister to *Senecio arnicoides* Hooker & Arnott (1830: 32), a species that occurs in central and southern Chile. In addition, *R. berteroi* was placed in a clade with *Robinsonia gracilis* Decaisne (1834: 29) in a plastid phylogeny (Pelser *et al.* 2007). However the plastid tree did not include the remaining *Robinsonia* species and had only a limited number of *Senecio* species represented. *Robinsonia berteroi* differs from the other *Robinsonia* species in having more deeply-lobed disk florets and more numerous capitula with fewer florets, but lacking ray florets. Because of these morphological differences, De Candolle (1833) considered this species different from *Robinsonia s. s.* and assigned it to the monotypic genus *Balbisia* De Candolle in Guillemain (1833: 333), which was subsequently changed by Meisner (1839) to *Rhetinodendron* Meisner (1839: vol. 1 216, vol. 2 136), because De Candolle's name proved to be a later homonym of *Balbisia* Cavanilles (1804: 61, Sanders *et al.* 1987). Sanders *et al.* (1987) considered the differences between *Rhetinodendron* and *Robinsonia* to be insufficient to maintain both genera as separate taxa, and combined them in *Robinsonia sensu lato* (Pacheco *et al.* 1985). Unfortunately, *Robinsonia berteroi* is believed to have gone extinct in 2004 (Danton & Perrier 2005).

While the monophyly of *Robinsonia s. l.* does not affect the generic delimitation of Senecioneae since all species of *Robinsonia* are better regarded as *Senecio* species (Pelser *et al.*, 2007), the question is relevant to studies on the biogeographic history and evolution of the Juan Fernández Islands flora. A polyphyletic *Robinsonia* would not only imply that *Senecio* lineages colonized the Juan Fernández archipelago twice, but also that the dioecious and tree-like habit evolved independently in two *Senecio* lineages in the same archipelago. A polyphyletic *Robinsonia* could also have consequences for molecular dating studies in Asteraceae and for other plant families since this genus is commonly used as a calibration point. *Robinsonia berteroi*, *R. evenia* Philippi (1856: 644), *R. gayana* Decaisne (1834: 28), *R. gracilis*, *R. macrocephala* Decaisne (1834: 28), *R. saxatilis* Danton (2006: 253) and *R. thurifera* Decaisne (1834: 28) are all endemic to the Isla Más a Tierra (= Robinson Crusoe Island), which is ca. 4 million years old. *Robinsonia masafuerae* Skottsberg (1922: 195) is an endemic of Isla Más Afuera (= Alejandro Selkirk Island), which is estimated to be 1–2.4 million years old (Stuessy *et al.* 1984). Sang *et al.* (1995) used these data to calculate the average ITS substitution rate within *Robinsonia*, assuming a maximum age of 4 million years for the genus. This estimate has been widely applied in molecular dating studies within *Senecio* (e.g., Comes & Abbott 2001, Coleman *et al.* 2003), in studies of additional Asteraceae (e.g., Liu *et al.* 2006), and other plant families (e.g., Bittkau & Comes 2005, Meister *et al.* 2006). Sang *et al.* (1995) estimated the average substitution rate of *Robinsonia* under the assumption that *Robinsonia* is monophyletic. If, however, this assumption proves to be incorrect, this would lower the estimate of the substitution rate of *Robinsonia* and therefore may affect age estimates for many studies.

The aim of this study was to assess the monophyly of *Robinsonia s. l.* and to identify its closest sister group. The phylogenies were reconstructed from DNA sequence data of two nuclear (ITS and ETS) and seven plastid markers (*ndhF* gene; *trnL* intron; *psbA-trnH*, *psbJ-petA*, 5' and 3' *trnK*, and *trnL-F* intergenic spacers). In addition, new names and combinations of the *Robinsonia s. l.* species under *Senecio* are presented.

## Materials and Methods

Taxon sampling, DNA extraction, amplification, and sequencing

Six of eight described species of *Robinsonia* were included in this study and were represented by ten accessions. Tissue samples of *R. saxatilis* (generously provided by Philippe Danton) did not yield DNA of sufficient quality, and DNA of *Robinsonia macrocephala* was unavailable to us. *Robinsonia saxatilis* is similar in morphology to *R. gayana* (Tod Stuessy, pers. comm.), and a cladistic analysis of morphology indicates that *R. macrocephala* is most closely related to *R. gayana* and *R. thurifera* (Sanders *et al.* 1987).

Thus the absence of these two taxa in our study is unlikely to significantly affect conclusions regarding the monophyly of *Robinsonia* since all three allied species mentioned above were included. In addition to sampling six *Robinsonia* species, we sequenced nine *Senecio* species that were previously found to be more closely related to *R. berteroi* than to the other *Robinsonia* species. We also sampled nine *Senecio* species suggested to be more closely related to *Robinsonia s. s.* than to *R. berteroi* (Pelser *et al.* 2007). In addition, we expanded our sampling of South American *Senecio* lineages with the addition of seven species not included in previous studies. Nine additional species were also included to represent all major *Senecio* lineages previously identified (Pelser *et al.* 2007). *Crassocephalum crepidioides* (Bentham in Hooker & Bentham 1849: 438) Moore (1912: 211) was selected as the outgroup because it is most closely related to the *Senecio* lineage (Pelser *et al.* 2007).

Total genomic DNA was isolated with the Qiagen DNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA) from fresh or silica-gel dried leaves of field-collected plants or from tissue taken from herbarium specimens (B, CONC, Instituto de Investigaciones Agropecuarias (INIA), L, MA, MEL, MJG, MU, S, U, UEC). Seven plastid markers (*5'trnK*, *3'trnK*, *ndhF*, *psbA-trnH*, *psbJ-petA*, *trnL* intron, and the *trnL-trnF* intergenic spacer), and two nuclear (ETS and ITS) regions were sequenced. Information on PCR primers is listed in Table 1. PCR products were cleaned with the QiaQuick PCR Purification Kit (Qiagen, Valencia, CA, USA) or the Promega Wizard SV Gel and PCR Clean-up system (Promega Corporation, Madison, WI, USA), labeled using the DYEnamic ET Terminator Cycle Sequencing Kit (GE Healthcare, Piscataway, NJ, USA) or the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA), and sequenced on ABI 310, 3130 or 3730 automated DNA sequencers at the Center for Bioinformatics and Functional Genomics at Miami University (Oxford, Ohio, USA). Forward and reverse sequences were obtained for all samples, and contigs were assembled and proofread using Sequencher 4.7 (GeneCodes, Ann Arbor, MI, USA). GenBank accession numbers of the DNA sequences used in this study are presented in Table 2. Sequences were manually aligned using Se-Al v.2.0a11 (Rambout 1996).

**TABLE 1.** Characters, nucleotide substitution models, and sources of primers.

	No. nucleotide characters	No. informative nucleotide characters	Substitution model	No. gaps	No. informative gaps	Total no. characters	Total no. informative characters	Source of primers
ITS	777	133 (17.1%)	GTR+I+G	26	11 (42.3%)	803	144 (17.9%)	White <i>et al.</i> 1990, Blattner 1999
ETS	455	77 (16.9%)	GTR+I+G	20	7 (35.0%)	475	84 (17.7%)	Baldwin & Markos 1998, Markos & Baldwin 2001, Bayer <i>et al.</i> 2002
ITS-ETS	1232	210 (17.0%)	GTR+I+G	46	18 (39.1%)	1278	228 (17.8%)	
<i>trnL-F</i>	930	18 (1.9%)	TVM+I	8	4 (50.0%)	938	22 (2.3%)	Taberlet <i>et al.</i> 1991
<i>psbA-trnH</i>	466	12 (2.6%)	HKY+I	24	10 (41.7%)	490	22 (4.5%)	Sang <i>et al.</i> 1997
<i>5' trnK</i>	558	8 (1.4%)	HKY+I	8	3 (37.5%)	566	11 (1.9%)	Pelser <i>et al.</i> 2002
<i>3' trnK</i>	583	7 (1.2%)	F81+I	11	7 (63.6%)	594	14 (2.4%)	Pelser <i>et al.</i> 2002
<i>ndhF</i>	1092	14 (1.3%)	F81+I	5	1 (20.0%)	1097	15 (1.4%)	Jansen, 1992, Pelser <i>et al.</i> 2007
<i>psbJ-petA</i>	901	14 (1.6%)	K81uf+G	11	5 (45.5%)	912	19 (2.1%)	Shaw <i>et al.</i> 2007
Plastid	4530	73 (1.6%)	K81uf+I	67	30 (44.8%)	4597	103 (2.2%)	
Total	5762	283 (4.9%)	TrN+I+G	113	48 (42.5%)	5875	331 (5.6%)	

**TABLE 2.** Voucher information and GenBank accession numbers.

Taxon	Voucher specimens	Distribution	ITS	ETS	<i>trnL</i> and <i>trnL-F</i>	<i>psbA-trnH</i>	3' <i>trnK</i>	5' <i>trnK</i>	<i>ndhF</i>	<i>psbI-petA</i>
<i>Crassocephalum crepidioides</i> (Benth.) S.Moore	<i>Pelser 354</i> (Cult; L)	Tropical Africa, Madagascar, Yemen	AF459968	GU818144	AF460138, EF028722	AY155640	AF459991	AF460050	EF537958	-
<i>Crassocephalum crepidioides</i> (Benth.) S.Moore	<i>B. Nordenstam &amp; R. Lundin 562</i> (S)	Tropical Africa, Madagascar, Yemen	EF538173	-	-	-	-	-	-	-
<i>Robinsonia berteroi</i> (DC.) R.W.Sanders, Stuessy & Martic.	<i>T. Stuessy et al. 11238</i> (CONC)	Juan Fernández	AF459957, EF028712, EF028719	GU818240	AF460129, EF538142	EF538082	AF459982	AF460040	EF537987	HM050343
<i>Robinsonia evenia</i> Phil.	<i>T. Stuessy et al. 11308</i> (CONC)	Juan Fernández	EF028706, EF028713	GU818241	GU818055	GU818432	GU817513	GU817680	GU817902	HM050345
<i>Robinsonia evenia</i> Phil.	<i>T. Stuessy et al. 11636</i> (CONC)	Juan Fernández	EF028707, EF028714	-	-	-	-	-	-	-
<i>Robinsonia gayana</i> Decne.	<i>T. Stuessy et al. 11285</i> (CONC)	Juan Fernández	EF028708, EF028715	HM050319	HM050371	HM050337	HM050311	HM050317	HM050329	HM050344
<i>Robinsonia gracilis</i> Decne.	<i>T. Stuessy, D. Crawford, H. Valdebenito &amp; A. Landero 6560</i> (B)	Juan Fernández	EF538290	GU818242	EF538118	EF538068	EF537933	EF042166	EF537988	-
<i>Robinsonia gracilis</i> Decne.	<i>T. Stuessy et al. 11312</i> (CONC)	Juan Fernández	EF028709, EF028716	GU818244	GU817946, GU817963	GU818434	GU817515	GU817681	-	HM050369
<i>Robinsonia gracilis</i> Decne.	<i>T. Stuessy et al. 11282</i> (CONC)	Juan Fernández	-	GU818243	GU817947, GU817962	GU818433	GU817514	-	GU817903	HM050368
<i>Robinsonia masafuerae</i> Skottsbo.	<i>Landero &amp; Ruiz 9301 and 9633</i> (CONC)	Juan Fernández	EF028710, EF028717	-	-	-	-	-	-	-
<i>Robinsonia masafuerae</i> Skottsbo.	<i>T. Stuessy et al. s.n.</i> (CONC)	Juan Fernández	-	GU818245	GU817945, GU817961	GU818435	GU817516	GU817682	GU817818	-
<i>Robinsonia thurifera</i> Decne.	<i>T. Stuessy et al. 11887</i> (CONC)	Juan Fernández	EF028711, EF028718	HM050320	HM050372	HM050338	HM050310	HM050313	HM050330	HM050346
<i>Senecio acanthifolius</i> Hombr. & Jacq., ex Decne.	<i>B. Björnsäter s.n.</i> (S)	Argentina, Chile	EF538238	GU818248	EF538104	EF538034	EF537923	EF042154	EF537970	HM050358
<i>Senecio algens</i> Wedd.	<i>S.G. Beck 2879</i> (S)	Bolivia, Chile	EF538296	GU818249	GU818058	GU818438	GU817519	GU817685	GU817905	HM050351
<i>Senecio arnicoides</i> Hook. & Arn.	<i>O. Zöllner 3474</i> (L)	Chile	EF538298	GU818250	GU818059	GU818439	GU817520	GU817686	GU817801	HM050359
<i>Senecio boyacensis</i> Cuatrec.	<i>J. Gonzalez 180</i> (S)	Bolivia	EF538176	GU818251	GU818060	GU818440	GU817521	GU817687	GU817906	HM050342
<i>Senecio calocephalus</i> Poepp.	M.F. Gardner, P. Hechenleitner V., C. Martinez A. & P.I. Thomas DCI no. 641 (CONC)	Chile	HM050325	HM050321	-	HM050340	HM050307	HM050314	HM050331	HM050347
<i>Senecio campanulatus</i> Sch.Bip. ex Klatt	<i>S.G. Beck &amp; R. Seidel 14415</i> (S)	Bolivia	EF538149	HM050324	HM050374	-	HM050305	HM050312	HM050328	HM050341
<i>Senecio candidans</i> DC.	<i>E. Pisano, Hendriquez &amp; Dominguez 7754</i> (CONC)	Argentina, Chile	HM050326	HM050322	HM050370 (trnL only)	-	HM050308	HM050315	HM050333	-
<i>Senecio chilensis</i> Less.	<i>O. Zöllner 2958</i> (L)	Chile	EF538313	GU818254	EF538122	EF538043	EF537936	EF042170	EF537992	HM050367
<i>Senecio cremeiflorus</i> Mattf.	<i>S.A. Renvoize 3378</i> (S)	Argentina	EF538320	HM050323	HM050375	HM050335	HM050306	-	-	-
<i>Senecio ctenophyllus</i> Phil.	<i>O. Zöllner 3959</i> (L)	Chile	EF538322	GU818255	GU818063	GU818443	GU817524	GU817690	GU817804	-
<i>Senecio culcitoides</i> Wedd.	<i>B. Øllgaard &amp; H. Balslev 8822</i> (U)	Ecuador	EF538312	GU818253	GU818062	GU818442	GU817523	GU817689	GU817907	-
<i>Senecio fistulosus</i> Poepp. ex Less.	<i>S.G. Beck &amp; M. Liberman 9672</i> (S)	Bolivia, Chile	EF538335	GU818258	GU818065	GU818445	GU817526	GU817692	GU817909	HM050363
<i>Senecio flaccidus</i> Less.	<i>Bain 521</i> (LEA)	USA	AF161640, AF161690	-	-	DQ131873	-	-	-	-

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TABLE 2 (continued)

Taxon	Voucher specimens	Distribution	ITS	ETS	<i>trnL</i> and <i>trnL-F</i>	<i>psbA-trnH</i>	3' <i>trnK</i>	5' <i>trnK</i>	<i>ndhF</i>	<i>psbI-petA</i>
<i>Senecio flaccidus</i> Less.	Jardin Thuret s.n. (Cult.; MJG)	USA	EF538336	GU818259	GU818066	-	GU817527	GU817693	GU817910	HM050354
<i>Senecio gayanus</i> DC.	M. Rosas 2157 (INIA)	Chile	GU818649	GU818261	GU818067	GU818446	GU817528	GU817694	GU817911	HM050349
<i>Senecio gregorii</i> F.Muell.	D.E. Albrecht 7091 (NT)	Australia	GU818651	GU818263	GU818069	GU818448	GU817530	GU817696	GU817912	HM050364
<i>Senecio grossidens</i> Dusén ex Malme	J.R. Trigo UEC 120.301 (UEC)	Brazil	EF538342	-	HM050376	HM050336	-	HM050316	HM050332	-
<i>Senecio hieracium</i> J.Rémy	M. Baeza & L. Finot 3695 (CONC)	Argentina, Chile	GU818652	GU818265	GU818070	GU818449	GU817531	GU817697	GU817913	HM050350
<i>Senecio hypsobates</i> Wedd.	B. Øllgaard & H. Balslev 9863 (U)	Ecuador	EF538348	GU818268	GU818073	GU818452	GU817533	GU817700	GU817822	HM050352
<i>Senecio integerrimus</i> Nutt.	S. Crockett 437 (MU)	USA	EF538349	GU818270	GU818075	-	GU817535	GU817702	GU817916	HM050353
<i>Senecio integerrimus</i> Nutt.	Golden 192 (LEA)	USA	-	-	-	DQ131871	-	-	-	-
<i>Senecio involucratus</i> (Kunth) DC.	B. Nordenstam 9438 (S)	Ecuador	EF538150	-	EF538090	EF538022	EF537910	EF042142	EF537952	-
<i>Senecio jarae</i> Phil.	M. Liberman L54 (S)	Argentina, Bolivia, Chile, Peru	EF538350	GU818271	EF538125	EF538044	EF537939	EF042175	EF537997	-
<i>Senecio lastarrianus</i> J.Rémy in Gay	M. Ricardi 3230 (B)	Chile	GU818663	GU818272	GU818076	GU818454	GU817536	GU817703	GU817823	HM050348
<i>Senecio mairetianus</i> DC.	J. Garcia P. 151 (L)	Mexico	EF538359	GU818275	EF538128	EF538045	EF537942	EF042178	EF538001	-
<i>Senecio nemorensis</i> L.	Pelser 102 (Cult.; L)	Europe	AF459937	GU818278	AF460150, EF028730	EF538046	AF460004	AF460064	EF538004	HM050360
<i>Senecio nemorensis</i> L.	Liu 1874 (HNWP)	Europe	-	-	-	-	-	-	AY723209	-
<i>Senecio niveo-aureus</i> Cuatrec.	A.M. Cleef 6665 (S)	Colombia	EF538178	GU818280	GU818078	GU818456	GU817538	GU817705	GU817918	-
<i>Senecio oerstedianus</i> Benth. ex Oerst.	B. Nordenstam 9160 (S)	Costa Rica	EF538362	GU818281	GU817950	GU818457	GU817539	GU817706	GU817919	HM050356
<i>Senecio oreophyton</i> J.Rémy	S.G. Beck 21589 (S)	Bolivia, Chile	EF538393	GU818282	GU818079	GU818458	GU817540	GU817707	GU817920	HM050355
<i>Senecio patens</i> (Kunth) DC.	V. Zak & J. Jaramillo 3427 (L)	Ecuador	EF538151	GU818284	GU818080	GU818459	GU817541	GU817708	GU817795	-
<i>Senecio pflanzii</i> (Perkins) Cuatrec.	S.G. Beck 9094 (S)	Bolivia	EF538179	GU818285	EF538096	EF538027	EF537916	EF042148	EF537960	-
<i>Senecio polygaloides</i> Phil.	O. Zöllner 5442 (L)	Chile	EF538367	GU818288	GU818082	GU818461	GU817543	GU817710	GU817923	HM050357
<i>Senecio suaveolens</i> (L.) Ell.	D.C. Dister s.n. 27 Jul 2002 (MU)	USA	EF538222	GU818298	EF538102	EF538032	EF537921	EF042152	EF537968	-
<i>Senecio suaveolens</i> (L.) Ell.	Botanischer Garten Potsdam 139 (Cult.; MJG)	USA	EF538223	-	-	-	-	-	-	-
<i>Senecio superandinus</i> Cuatrec.	F. Hekker & W.H.A. Hekking 10.159a (U)	Ecuador	EF538248	GU818299	EF538105	EF538035	EF537924	EF042155	EF537973	-
<i>Senecio trifurcatus</i> (G.Forst.) Less.	M. Ricardi, C. Marticorena & O. Matthei 1838 (B)	Chile	HM050327 (only ITS1)	-	HM050373	HM050339	HM050309	HM050318	HM050334	-
<i>Senecio triodon</i> Phil. var. <i>triodon</i>	F. Luebert & S. Teillier 2266 (CONC)	Chile	GU818707	GU818303	GU818088	GU818468	GU817550	GU817717	GU817930	HM050362
<i>Senecio viscosus</i> L.	Pelser 300 (L)	Europe	AF459925	GU818306	AF460142, EF028734	AY155666	AF459996	AF460055	EF538016	HM050366
<i>Senecio viscosus</i> L.	R.I. Milne s.n. (STA)	Europe	AJ400808	-	-	-	-	-	-	-
<i>Senecio viscosus</i> L.	Vitek, Blab & Dietrich 50-2, 450 (MO)	Europe	AF097539	-	-	-	-	-	-	-

## ITS cloning

In order to assess whether the incongruence between the ITS-ETS and plastid trees for the phylogenetic position of *R. berteroi* is due to the presence of divergent ITS-ETS copies, ITS PCR products of *R. berteroi* were cloned and sequenced. The ETS region was not cloned, because the ITS and ETS trees were not strongly incongruent and both regions are adjacent. Cloning was performed using the TOPO TA Cloning Kit for Sequencing (Invitrogen, Carlsbad, CA, USA). A total of 11 clones were PCR-amplified directly from plated culture with the manufacturer's supplied M13 plasmid primers and sequenced. Sequencing and alignment followed the protocol outlined above.

## Phylogenetic analysis

For several species, sequences of multiple accessions were available (Table 2). In a first set of heuristic searches under maximum parsimony (MP, see below), all available sequences were included. When multiple accessions of the same species formed a monophyletic lineage and sequence similarity was high, a consensus sequence was generated for subsequent phylogenetic analyses in which ambiguous bases were coded as polymorphic (Pelsler *et al.* 2007, 2010). This strategy was chosen to include all available data potentially contributing to the phylogeny reconstruction of a taxon. A Python script (Richard Ree, Field Museum, Chicago) was used to code indels as binary characters using the 'simple indel coding method' of Simmons & Ochoterena (2000).

MP analyses were carried out using TNT 1.0 (Goloboff *et al.* 2008) using all 'New Technology' options in a 'Driven' search (i.e., sectorial search, ratchet, drift, and tree fusion) with 100 initial addition sequences, terminating the search after finding minimum length trees five times. Bootstrap values (Felsenstein 1985) were calculated from 1,000 replicates with TNT using a Driven search and Poisson independent reweighting. Bayesian Inference analyses (BI) were performed using the parallel version of MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003) on the Redhawk Cluster at Miami University. Parameters for the nucleotide substitution model were determined using the Akaike Information Criterion in Modeltest 3.7 (Posada & Crandall 1998, Table 1). Indel characters were included as 'restriction type' data in the BI analyses. Using random starting trees, MrBayes was run until the average standard deviation of the split frequencies of two simultaneous runs reached 0.01. One tree was sampled every 1,000 generations. The analyses were performed with 24 chains per run and a temperature setting of 0.01. Post-analysis was carried out in MrBayes to determine the number of trees to omit as 'burn in', and to compute the consensus tree and posterior probabilities. Maximum likelihood (ML) analyses were carried out with GARLI 0.96 (Zwickl 2008) using the substitution models selected with Modeltest (Table 1) and excluding indels. All other settings were kept as the defaults, and each analysis was run five times. ML bootstrap analyses with 1,000 replicates were conducted with GARLI on the Cimarron Cluster at Oklahoma State University.

Data sets of the individual nuclear and plastid DNA regions were analyzed both separately and combined. Incongruence length difference tests (ILD, Farris *et al.* 1995) using PAUP\* 4.0b10 (Swofford 2003) were performed to test for congruence among these regions. The ILD tests were implemented using 1,000 replicates with 10 random addition sequences per replicate with maxtrees settings between 2,500 and 100,000 trees depending on the size and complexity of the data. Following Cunningham (1997), p-values below 0.01 were considered significant. Because this test has been shown to suffer from type I errors when phylogenetic signal is low (Yoder *et al.* 2001, Hipp *et al.* 2004), phylogenies of the individual DNA regions were compared to each other to detect areas of well-supported incongruence (i.e., differences supported by high bootstrap values and/or posterior probabilities; Seelanan *et al.* 1997). Incongruent taxon placements among phylogenies were only considered significant if support values were equal to or above a bootstrap value of 80% or a posterior probability of 0.95.

To test the monophyly of *Robinsonia s. l.*, Shimodaira-Hasegawa tests (SH, Shimodaira & Hasegawa 1999) were performed on the ITS-ETS, plastid, and combined data sets. SH tests were carried out with PAUP\* (Swofford 2003) using RELL bootstrapping with 1,000 replicates. Alternative topologies obtained from ML analyses in which *Robinsonia s. l.* was constrained to be either monophyletic or non-monophyletic were compared to trees reconstructed in unconstrained MP, BI, and ML analyses.

## Results

### Plastid data

Phylogenies obtained from individual plastid markers were incompletely resolved due to an insufficient number of characters (Table 1). The ILD tests did not indicate significant incongruence among the plastid regions ( $p = 0.61$ ), and a comparison of support values of incongruent clades did not reveal well-supported conflict between trees obtained from the individual plastid regions. We therefore performed all subsequent analyses of the combined plastid data. MP, BI, and ML analyses of this combined data set resulted in trees that are similar with the exception of weakly-supported clades and minor differences in resolution. All plastid trees resolved *Robinsonia* as monophyletic with high support in the BI tree (Fig. 1a;  $pp=1.0$ ) but with low bootstrap support in the MP (<50%) and ML (64%) analyses. SH tests comparing unconstrained MP, BI, and ML trees with an ML tree in which *Robinsonia* was forced to be non-monophyletic, did not reveal any significant differences in likelihood values between these alternative topologies (Table 3).

**TABLE 3.** Results of the Shimodaira-Hasegawa (SH) constraint analyses. All MP trees, the MP majority rule consensus tree, the BI consensus tree, ten trees with the highest posterior probabilities from the BI analyses, and the most likely trees from ML analyses were compared to ML trees in which *Robinsonia* was constrained to be non-monophyletic (plastid data set and combined data set) or monophyletic (ITS-ETS data set and combined data set).

Trees	No. of trees	p =
Plastid data set:		
MP trees	36	0.256–0.993
MP consensus	1	0.174
BI trees	10	best & 0.492–1.000
BI consensus	1	1.00
ML trees	1	0.986
ML constraint tree: <i>Robinsonia</i> non-monophyletic	1	0.574
ITS-ETS data set:		
MP trees	72	0.327–0.962
MP consensus	1	0.402
BI trees	10	0.641–0.977
BI consensus	1	0.965
ML trees	1	best
ML constraint tree: <i>Robinsonia</i> monophyletic	1	0.894
Combined data set:		
MP trees	39	0.421–0.804
MP consensus	1	0.525
BI trees	10	0.913–0.953
BI consensus	1	0.874
ML trees	1	best
ML constraint tree: <i>Robinsonia</i> monophyletic	1	0.953
ML constraint tree: <i>Robinsonia</i> non-monophyletic	1	0.836

### ITS-ETS data

A visual comparison of ITS and ETS topologies and an ILD test failed to reveal significant incongruence between the two data sets ( $p = 0.156$ ). Thus additional analyses of the two markers were carried out on a combined data set. All phylogenetic analyses (MP, BI, and ML) of this combined ITS-ETS data set failed to

resolve *Robinsonia* as monophyletic (Fig. 1b), with *R. berteroi* placed within a clade composed of primarily southern South American species (the *S. acanthifolius* Hombron & Jacquinot ex Decaisne (1853: 46) - *S. fistulosus* Poeppig ex Lessing (1831: 246) clade). However, the placement of *R. berteroi* outside of the *Robinsonia* clade is only supported with a low bootstrap value (<50%) and low posterior probability ( $p = 0.63$ ). When *Robinsonia* was constrained to be monophyletic, no significant differences in likelihood values were apparent in the SH tests (Table 3).

A total of 10 of the 11 sequenced ITS clones of *R. berteroi* were identical or only different in a few base pair positions. An MP analysis in which these sequences and directly sequenced ITS products of all other taxa were included (results not shown) showed that all ITS copies of *R. berteroi* formed a monophyletic group. One ITS clone had an unusual ITS2 sequence that did not resemble the other ITS sequences included in our analyses nor sequences available from GenBank. This sequence was therefore omitted from the analyses.

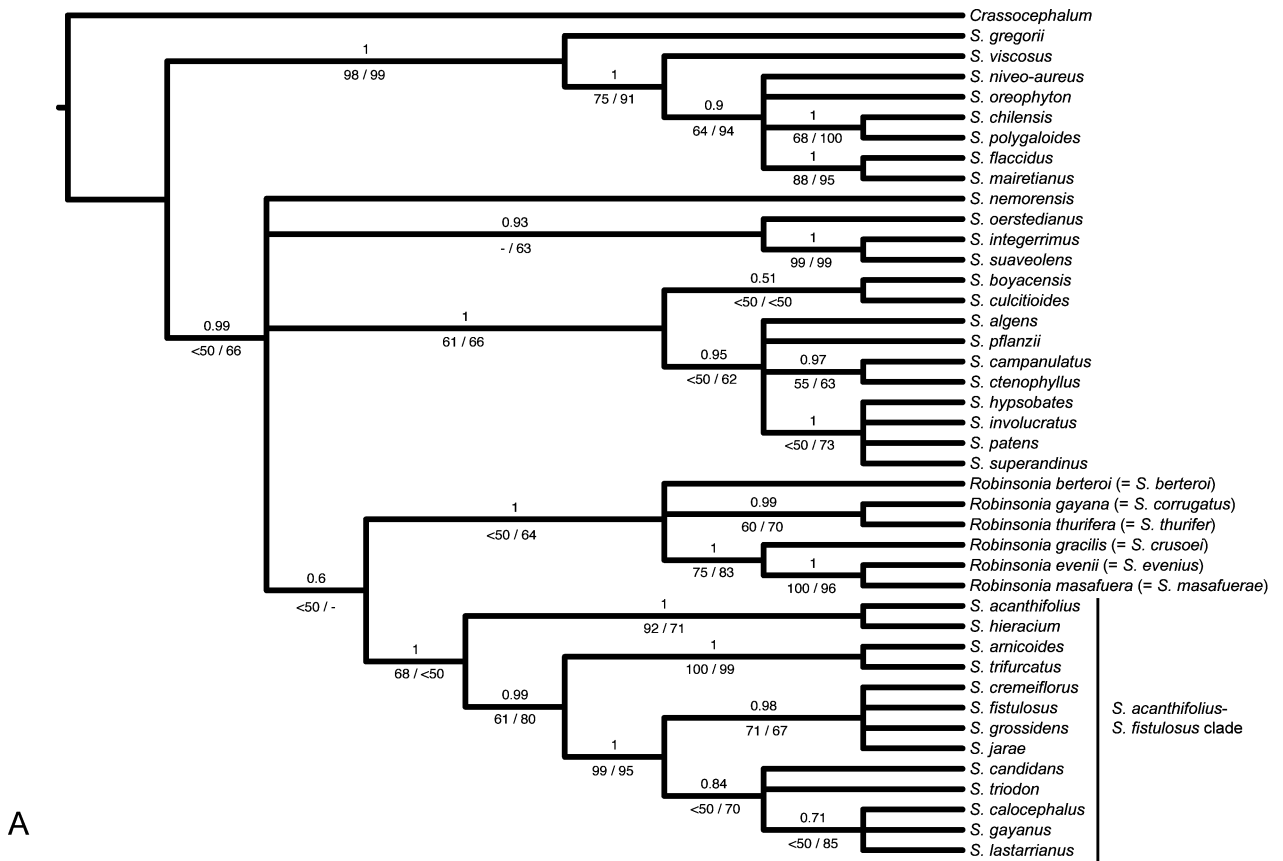
#### Incongruence between plastid and ITS-ETS data

An ILD test implemented to examine congruence between the ITS-ETS and combined plastid data sets resulted in a value of  $p = 0.009$ . A comparison of the BI topologies of these data sets further shows well-supported ( $p > 0.95$ ) incongruence regarding the phylogenetic positions of *S. candidans* (Vahl 1794: 91) De Candolle (1838: 412) and *S. campanulatus* Schultz ex Klatt (1888: 126). Whereas *S. candidans* forms a clade with *S. cremeiflorus* Mattfeld (1933: 325), *S. grossidens* Dusén ex Malme (1933: 101), and *S. fistulosus* in the ITS-ETS trees ( $p = 0.95$ ; Fig. 1b), plastid data suggest that *S. jarae* Philippi (1891: 44) is more closely related to these three species instead ( $p = 0.98$ ; Fig. 1a). Additionally, *S. candidans* is closer to *S. calocephalus* Poeppig (1845: 58), *S. gayanus* De Candolle (1838: 414), *S. lastarrianus* Rémy in Gay (1849: 146), and *S. triodon* Philippi (1858: 749), although the affinity with these species is only weakly-supported ( $p = 0.84$ ). *Senecio campanulatus* is the well-supported sister taxon of a clade composed of *S. involucratus* (Kunth in Bonpland *et al.* 1820: 166) De Candolle (1838: 422) and *S. patens* (Kunth in Bonpland *et al.* 1820: 164) De Candolle (1838: 423) in the ITS-ETS BI trees ( $p = 0.97$ ; Fig. 1b), but is placed sister to *S. ctenophyllus* Philippi (1891: 45) in the plastid trees ( $p = 0.97$ ; Fig. 1a). Also the MP and ML trees indicate incongruence regarding the phylogenetic positions of *S. candidans* and *S. campanulatus*, although this conflict is not supported by bootstrap values >80% (Fig. 1). In addition, the MP trees show incongruence with respect to the relationships between *S. gregorii* Von Mueller (1859: 14) and the other *Senecio* species. This species is sister to a moderately-supported (76% bootstrap support) clade composed of all other *Senecio* species in the ITS-ETS MP trees, whereas it is well-supported as a member of a clade formed by *S. chilensis* Lessing (1831: 248), *S. flaccidus* Lessing (1830: 161), *S. mairetianus* De Candolle (1838: 430), *S. niveo-aureus* Cuatrecasas (1940: 6), *S. oreophyton* Rémy in Gay (1849: 158), *S. polygaloides* Philippi (1894: 32), and *S. viscosus* Linnaeus (1753: 868) in the plastid bootstrap consensus tree (98% bootstrap support; Fig. 1a). An ILD test performed on the combined plastid-ITS-ETS data set from which *S. candidans* and *S. campanulatus* were excluded resulted in  $p = 0.023$ . A value of  $p = 0.24$  resulted when, in addition to these two species, *S. gregorii* was excluded.

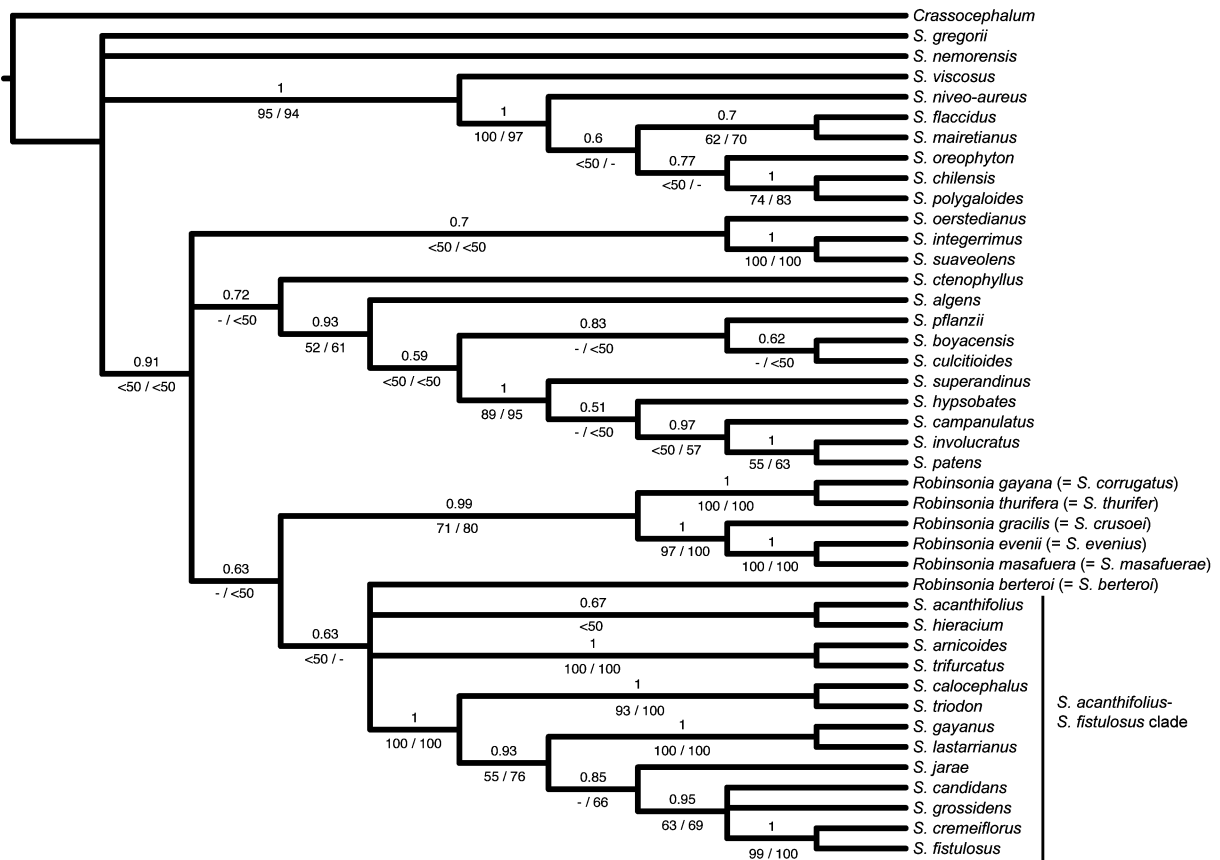
#### Combined plastid-ITS-ETS data set

Even though the results of the BI analyses provide well-supported incongruence between the ITS-ETS and plastid data sets, analyses of a combined plastid-ITS-ETS data set were performed to examine the monophyly of *Robinsonia s. l.* This was done because the taxa that are found in well-supported incongruent phylogenetic positions are relatively distantly related to the *Robinsonia* species, and the incongruent patterns do not appear to affect conclusions regarding the monophyly of *Robinsonia*. The results of MP and BI analyses indicate that *Robinsonia s. l.* is monophyletic (Fig. 2), whereas a non-monophyletic *Robinsonia* was resolved in the ML trees (not shown). Both topological hypotheses are however only weakly-supported, and alternative topologies in which *Robinsonia s. l.* was either constrained to be monophyletic or to be non-monophyletic did not have significantly lower likelihood values than trees obtained in unconstrained analyses (Table 3).



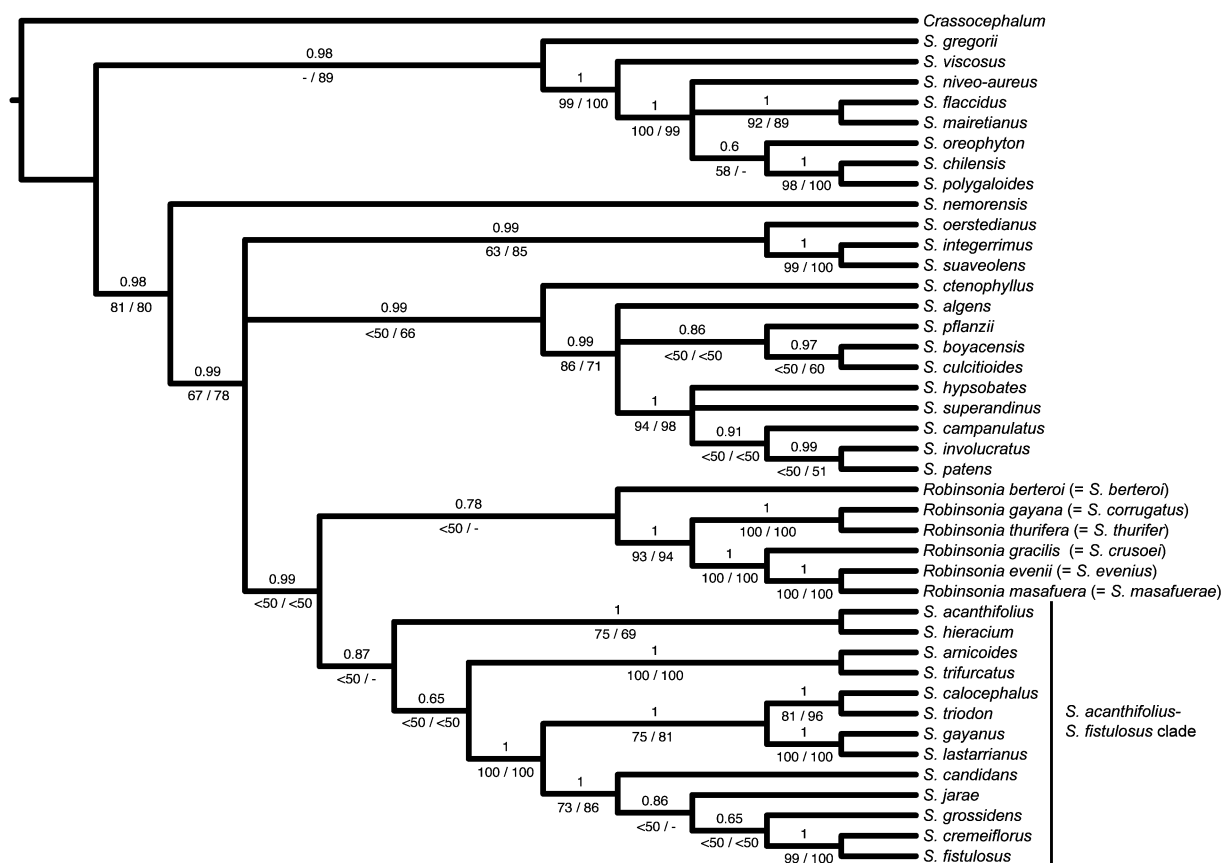


A



B

**FIGURE 1.** Bayesian consensus trees from: A, plastid DNA sequences (*ndhF* gene; *trnL* intron; *psbA-trnH*, *psbJ-petA*, 5' and 3' *trnK*, and *trnL-F* intergenic spacers) and B, ITS-ETS sequences. Posterior probabilities are shown above the branches. MP bootstrap values precede ML bootstrap values below the branches.



**FIGURE 2.** Bayesian consensus tree from a combined plastid-ITS-ETS data set (plastid and ITS-ETS data). Posterior probabilities are shown above the branches. MP bootstrap values precede ML bootstrap values below the branches.

## Discussion

The results of the phylogenetic analyses of ITS-ETS and plastid DNA sequence data are inconclusive regarding the monophyly of *Robinsonia s. l.* Although the plastid data (Fig. 1a) and the results of the MP and BI analyses of the combined plastid-ITS-ETS data set (Fig. 2) indicate that *Robinsonia s. l.* is monophyletic, this result is only well-supported in the BI trees of the plastid data set ( $p = 1.00$ ). In contrast, the ITS-ETS trees (Fig. 1b) and the ML tree obtained from the combined plastid-ITS-ETS data set (not shown) suggest, with low bootstrap support and posterior probabilities, that *Robinsonia s. l.* is not monophyletic and that *R. berteroi* is more closely related to species of a South American *Senecio* clade (the *S. acanthifolius-S. fistulosus* clade). These findings are supported by the SH tests, which reveal that trees from reciprocal analyses in which *Robinsonia s. l.* was constrained to be monophyletic or to be non-monophyletic do not have significantly lower likelihood scores than trees from unconstrained analyses.

The failure to show conclusively whether *Robinsonia s. l.* is monophyletic or not is perhaps due to a lack of informative characters, which is especially a concern in the plastid data set (Table 1). This may also be an explanation for the incongruence observed between the ITS-ETS and plastid trees. In addition to the weakly-supported topological conflict regarding the monophyly of *Robinsonia s. l.*, well-supported incongruence between the ITS-ETS and plastid BI trees was found, which affects the phylogenetic positions of *S. candidans* and *S. campanulatus*. This incongruence could signal, amongst others, ITS/ETS orthology/paralogy confusion (Doyle 1992, Álvarez & Wendel, 2003) or differences in the evolutionary histories of DNA regions (i.e., gene tree – species tree discordance) resulting from hybridization or incomplete lineage sorting (Doyle 1992, Maddison 1997, Buckley *et al.* 2006, Liu & Pearl 2007). Although studies using more variable plastid and single- or low copy nuclear markers need to be undertaken to distinguish between these alternative

hypotheses, incomplete lineage sorting seems to be relatively unlikely as an explanation for the incongruent phylogenetic position of *R. berteroi*. *Robinsonia s. l.* is endemic to the Juan Fernández Islands and therefore if incomplete lineage sorting occurred, ancestral polymorphisms must have survived the bottleneck in population size associated with the colonization of this island group. Because ITS cloning experiments did not reveal strongly divergent ITS copies, ITS-ETS orthology/paralogy confusion also does not seem to be a likely explanation for the incongruent position of *R. berteroi*. If the topological conflict with regards to the monophyly of *Robinsonia s. l.* resulted from hybridization, this may have occurred between a *Robinsonia* species and a member of the largely southern South American *S. acanthifolius*-*S. fistulosus* clade, which is sister to *Robinsonia s. s.* or *s. l.* in most plastid, ITS-ETS, and combined plastid-ITS-ETS trees. This scenario would imply that the Juan Fernández archipelago has been colonized by *Senecio* lineages twice: once by the ancestor of *Robinsonia* and once by a member of the *S. acanthifolius*-*S. fistulosus* clade which is now extinct on the islands. A hybrid origin of *R. berteroi* or introgression with a now extinct hybrid between *Robinsonia* and the *S. acanthifolius*-*S. fistulosus* clade may also explain the rather different floral morphology of *R. berteroi* compared to the other *Robinsonia* species. Aside from floral characters, *R. berteroi*, however, closely resembles *Robinsonia s. s.* and lacks obvious morphological similarity to members of *Senecio* lineages including the *S. acanthifolius*-*S. fistulosus* clade. Due to the weakly-supported phylogenetic position of *R. berteroi* within the *S. acanthifolius*-*S. fistulosus* clade in the ITS-ETS and ML combined plastid-ITS-ETS trees, it is unclear which lineage within this clade may have contributed to the genome of *R. berteroi*.

## Taxonomy

The results of the present paper corroborate those of Pelser *et al.* (2007, 2010) in showing that *Robinsonia* is nested within *Senecio*. We here propose to transfer the species of *Robinsonia* to *Senecio*, which requires the following new names and combinations.

### *Senecio berteroi* (De Candolle) Pelser, *comb. nov.*

Basionym: *Balbisia berteroi* De Candolle in Guillemin (1833: 333). Type: *Bertero 1467* (isotype? K!), as '*Berterii*'.

Homotypic synonyms: *Vendredia berterii* (De Candolle) Baillon (1881: 264).

*Rhetinodendron berteroi* (De Candolle) Hemsley (1884: 39).

*Ingenhousia berteroi* (De Candolle) Kuntze (1891: 348).

*Robinsonia berteroi* (De Candolle) Sanders, Stuessy & Marticorena in Stuessy & Marticorena (1990: 79).

Nomen nudum: *Ingenhousia thurifera* Bertero ex Hemsley (1884: 39), *pro syn.*

### *Senecio corrugatus* (Philippi) Pelser, *comb. nov.*

Basionym: *Robinsonia corrugata* Philippi (1856: 645). Type: *Philippi s.n.* (B, lost); neotype: October 1881, *Germain s.n.* (SGO!, designated here following Muñoz Pizarro (1960)).

Heterotypic synonyms: *Robinsonia gayana* Decaisne (1834: 28). Type: *Decaisne s.n.* (holotype BR!).

*Robinsonia longifolia* Philippi (1856: 644). Type: *Philippi 557* (B, lost); neotype: October 1854, *Germain s.n.* (SGO!, designated here following Muñoz Pizarro (1960)).

Note:—The names *Senecio gayanus* and *Senecio longifolius* are not available (*Senecio gayanus* De Candolle (1838: 414); *Senecio longifolius* Linnaeus (1763: 1222)).

***Senecio crusoei* Pelsner, *nom. nov.***

Nomen novum for *Robinsonia gracilis* Decaisne (1834: 29). Type: Genus forte distinctum, vulgo resinillo, in sylvaticis frigidis montium editiorum ad cacumen, May 1830, *Bertero 1510* (holotype BR!, isotypes G! K!).

Nomina nuda: *Senecio stenophyllus* Bertero ex Decaisne (1834: 29), *pro syn.*; *non Senecio stenophyllus* Philippi (1858: 743), *nec Senecio stenophyllus* Greenman (1907: 20).

*Robinsonia micrantha* Philippi ex Hemsley (1884: 41), *pro. syn.*

Note:—The names *Senecio gracilis* and *Senecio micranthus* are not available (*Senecio gracilis* Pursh (1814: 529); *Senecio micranthus* Hornemann (1819: 97)).

***Senecio evenius* (Philippi) Pelsner, *comb. nov.***

Basionym: *Robinsonia evenia* Philippi (1856: 644). Type: *Philippi 559* (B, lost); neotype: October 1854, *Germain, s.n.* (SGO!, designated here following Muñoz Pizarro (1960)).

***Senecio masafuerae* (Skottsberg) Pelsner, *comb. nov.***

Basionym: *Robinsonia masafuerae* Skottsberg (1922: 195). Syntypes: 15 February 1917, *C. & I. Skottsberg 434, female* (GB!, K!, SGO!); *C. & I. Skottsberg 1233, male* (GB!, SGO!).

Note:—The syntypes in GB are mounted on the same sheet and the whole should be considered for the interpretation of the species. For this reason it does not seem sensible to choose one specimen over the others, but in case this is demanded for any purpose, we suggest to treat the female specimen (*Skottsberg 434*, GB) as the lectotype (here designated).

***Senecio masatierrae* Pelsner, *nom. nov.***

Nomen novum for *Robinsonia macrocephala* Decaisne (1834: 28). Type: *Gay ?* (P).

Homotypic synonym: *Symphyochaeta macrocephala* (Decaisne) Skottsberg (1951: 785).

Note:—The name *Senecio macrocephalus* is not available (*Senecio macrocephalus* De Candolle (1838: 407)).

***Senecio petrophyus* Pelsner, *nom. nov.***

Nomen novum for *Robinsonia saxatilis* Danton (2006: 253). Type: 26 January 1999, *Danton B(665)632* (holotype SGO); *Danton B(665)632bis* (isotype P), *Danton B(665)632ter* (isotype Danton private herbarium).

Note:—The name *Senecio saxatilis* is not available (*Senecio saxatilis* Wallich ex De Candolle (1838: 367)).

***Senecio thurifer* (Decaisne) Pelsner, *comb. nov.***

Basionym: *Robinsonia thurifera* Decaisne (1834: 28). Type: Genus forte distinctum, vulgo resino, in sylvaticis petrosis mont. editiorum, April 1830, *Bertero 1511* (holotype G!, isotype K!).

Nomen nudum: *Senecio thurifer* Bertero ex Decaisne (1834: 28), *pro syn.*

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